Supplemental Material:

Cell types, isolates, and reagents used:

Vero E6 (ATTC CRL-1586) cells were maintained at 37°C with 5% CO_2 in Dulbecco's Modified Eagle Medium (Sigma), 10% Foetal Bovine Serum (HyClone) and 1% Penicillin/Streptomycin (VWR). SARS-CoV-2 Isolate USA-WA1/2020, NR-52281, was used for inactivation assays (deposited by the Centers for Disease Control and Prevention of the United States and obtained through BEI Resources, NIAID). Virus stocks were prepared by infecting Vero E6 cells at a multiplicity of infection (MOI) of 0.1 and collecting the supernatant at 3 days post-infection (dpi), and the concentration of virus present was determined by plaque assay at 3 dpi. For plaque assays, 4×10^5 Vero E6 cells per well were seeded in 12-well plates a day prior to infection, allowing for 95% confluence at the time of infection. Example photographs of the plaques and cell layers observed are included in Figure 1.

Neutralizer was prepared on the same day it was used, and our neutralizer recipe was adapted from Wood et al with two modifications (1). In our preparation, we used foetal bovine serum instead of horse serum, and we used 0.5 M Tris buffered at pH 7.5 instead of 0.25 M Tris buffered at pH 7.2. Neutralizer was passed through a 0.45 µm filter to sterilize.

Standard protocols used:

The test method was derived, with some adaptation, from ASTM protocol E1052-20, ASTM protocol E2197-19, and AOAC protocol 960.09 (2, 3, 4). The general form of this experiment, including the dilution scheme, neutralizer control, and bovine serum albumin soil load, come from ASTM E1052-20. The hard water used for this experiment

25 is 300 ppm $CaCO_3$, which is suggested in the ASTM E2197-19 protocol, while the

26 recipe used to make the hard water comes from AOAC protocol 960.09.

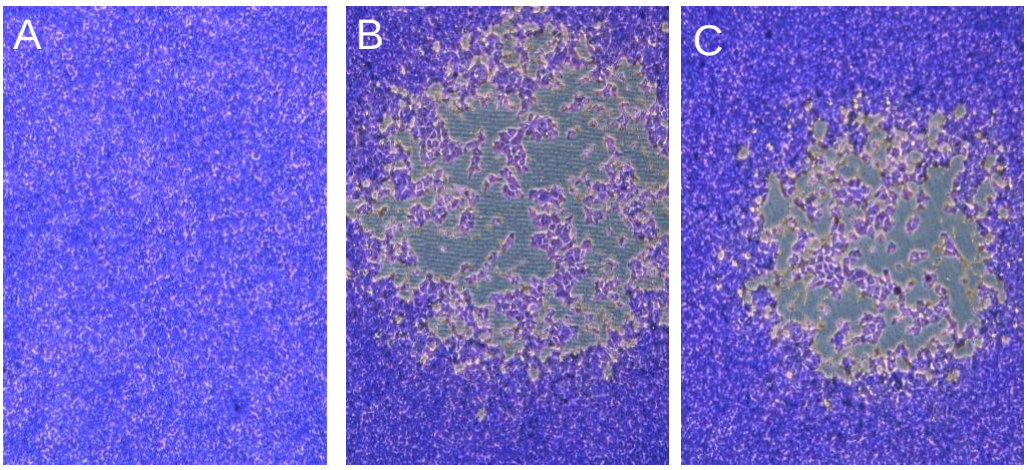

Figure 1: Micrographs of an intact cell layer (A), as well as two plaques (B, C). 20x magnification was used.

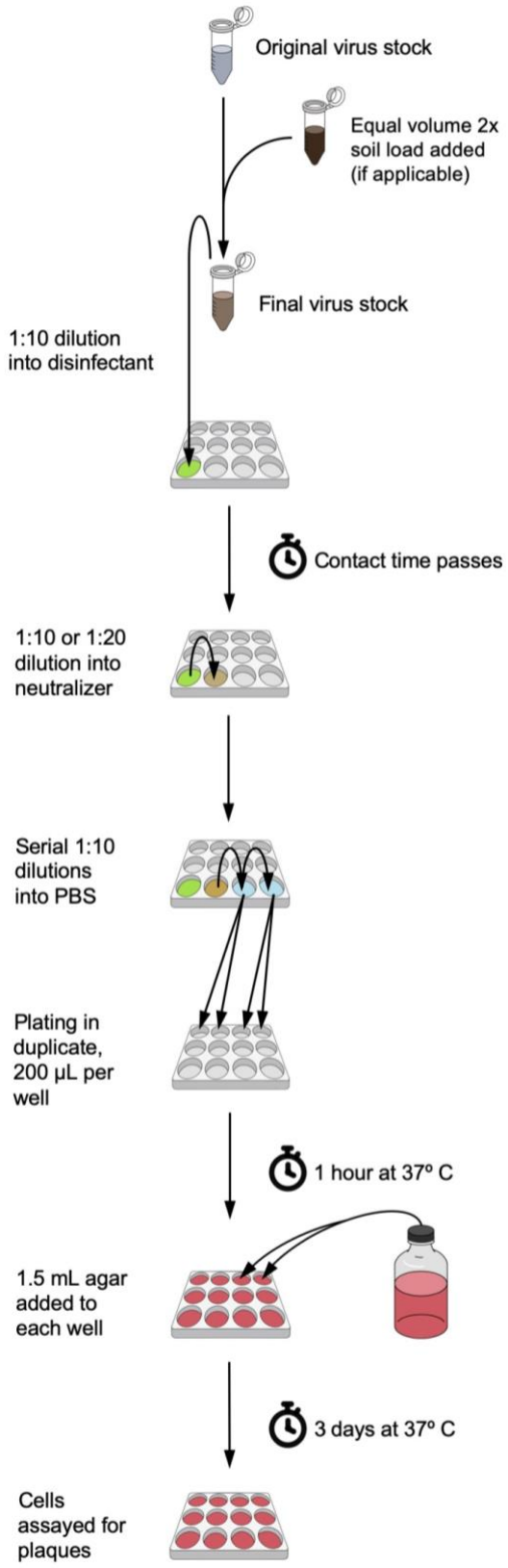

Figure 2: Diagram of test method.

References:

(1) Wood A, Payne D. The action of three antiseptics/disinfectants against enveloped and non-enveloped viruses. Journal of Hospital Infection 1998;38(4):283-295.

(2) AOAC 960.09-2011 Germicidal and Detergent Sanitizing Action of Disinfectants. 2011.

(3) ASTM 1053-20: Standard Practice to Assess Virucidal Activity of Chemicals Intended for
Disinfection of Inanimate, Nonporous Environmental Surfaces. 2020 Mar 11.

(4) ASTM E2197-17e1: Standard Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals. 2017.